



# Multifactorial comparison of photobioreactor geometries in parallel microalgae cultivations



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## ABSTRACT

Efficient photosynthetic biomass production in a high rate pond (HRP) or a photobioreactor (PBR) represents the first step of microalgae platforms for the production of renewable fuels, animal feeds and a diverse range of high value products. This study analyses the interplay between solar energy input, ambient temperature and system surface area to volume (SA:V) ratio in terms of photosynthetic performance (yield, areal and volumetric productivity, photon conversion efficiency). Ten pilot scale trials were conducted under subtropical conditions using 2 microalgae strains (*Chlorella sorokiniana* and *Chlorella* sp.) in 5 different cultivation system geometries: HRPs, flat panel PBRs (0.75 m and 1.5 m high) and tubular PBRs (0.74 m and 1.49 m high). The evaluation of culture temperature and biomass productivity response to solar irradiance in the five production systems suggests that the optimal SA:V ratio range lies between 43–73 m<sup>2</sup> m<sup>-3</sup> for *C. sorokiniana* in non-cooled systems regardless of system geometry under the conditions tested. The overall photosynthetic performance at higher SA:V ratios was improved for *Chlorella* sp. using temperature regulation. The highest observed daily photon conversion efficiency (PCE) was 4.44% (based on illuminated PBR surface area and total solar spectrum) in the high flat panel PBR using *C. sorokiniana* (40.8 g m<sup>-2</sup> d<sup>-1</sup>, 0.23 g L<sup>-1</sup> d<sup>-1</sup>). The highest achieved mean PCE (based on illuminated PBR surface area and total solar spectrum) was 2.5% in the low tubular PBR with *Chlorella* sp. (24.9 g m<sup>-2</sup> d<sup>-1</sup>, 0.43 g L<sup>-1</sup> d<sup>-1</sup>). The trial data provides important design principles to help fast track systems optimisation for near optimal sub-tropical conditions.

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## 1. Introduction

Microalgae provide a powerful biotechnology production platform for a broad range of products including renewable fuels [1], animal and fish feeds [2] and a diverse range of high value products [3–6]. The first step of all of these processes is the photosynthetic production

of biomass. Efficient biomass production requires high performance strains, nutrient and CO<sub>2</sub> sufficiency [7] as well as optimal light delivery to drive photosynthesis and optimal system temperature [8], which influences enzyme kinetics (Fig. 1). The development of high efficiency, low cost systems closely matching the needs of the cells is therefore a major focus of microalgae research and a challenging systems

**Abbreviations:** A<sub>footprint</sub>, bioreactor footprint area; A<sub>illuminated</sub>, illuminated area of a PBR; BOM, Bureau of Meteorology; CAPEX, capital expenditure; CU, control units; DW, dry weight; E<sub>biomass</sub>, energy content of biomass; EDTA, ethylene diamine tetra acetic acid; E<sub>PAR</sub>, mean daily energy received on a surface (MJ m<sup>-2</sup>) in the PAR region; FACS, fluorescence activated cell sorting; FC, flow cytometry; FP, flat panel PBR; HP strain, high performance strain *Chlorella* sp. (11\_H5); HRP, high-rate-pond; KBR, Kellogg Brown & Root Pty Ltd Australia; KIT, Karlsruhe Institute of Technology; LCA, life-cycle analysis; LC, low chlorophyll; LDPE, low density polyethylene; LP670-A, chlorophyll fluorescence filter area; NPQ, non-photochemical quenching; OPEX, operating expenditure; PBR, photo-bioreactor; PBS, phosphate buffered saline; P<sub>areal</sub>, areal biomass productivity (g m<sup>-2</sup> d<sup>-1</sup>); P<sub>vol</sub>, volumetric biomass productivity (g L<sup>-1</sup> d<sup>-1</sup>); PAR, photosynthetically active radiation; PCE, photon conversion efficiency; PFD, photon flux density; PVC, polyvinyl chloride; SD, standard deviation; rDNA, ribosomal DNA; REF strain, reference strain *Chlorella sorokiniana* (12\_A9); RT, room temperature; SA:V ratio, illuminated surface area to volume ratio; SBRC, Solar Biofuels Research Centre; TAP, Tris acetate phosphate medium; TEA, techno-economic analysis; UV, ultraviolet; vvm, gas volume flow per unit of liquid volume per minute; V<sub>reactor</sub>, reactor volume (L); Y<sub>vol</sub>, volumetric biomass yield (g L<sup>-1</sup>).

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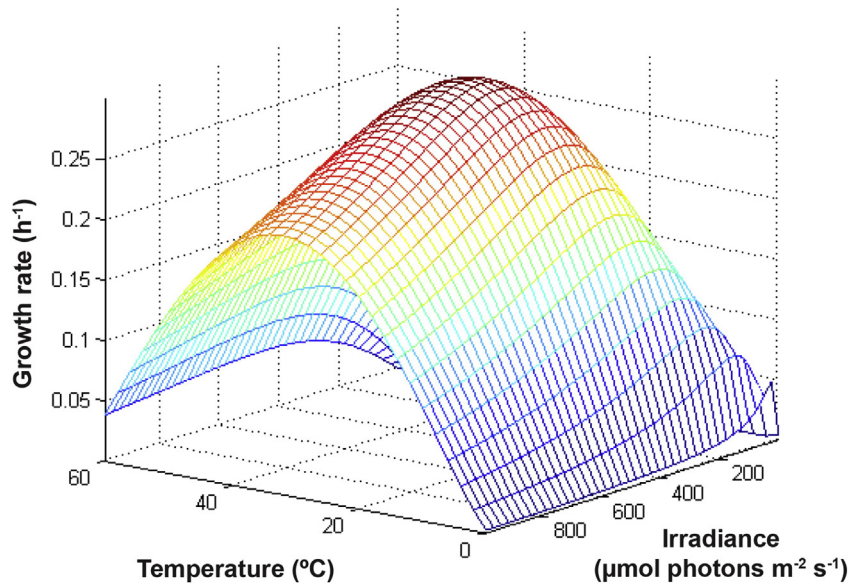


Fig. 1. Conceptual change in growth rate of microalgae in response to local PAR irradiance and culture temperature.

optimisation problem involving a large number of engineering variables (e.g. light dilution, surface area to volume (SA:V) ratio) and the provision of biologically optimal production conditions (e.g. temperature, nutrient supply) [9]. Increasing SA:V ratio is central to this process as it influences incident light delivered into the culture. However, it also affects system temperature and cost.

Here we report 10 pilot scale trials using two high performance algae strain isolates and five different production systems (high rate ponds, low and high flat panel and low and high tubular PBRs) under sub-tropical Australian conditions, which are well suited to commercial algae production due to their high solar energy density [10], year round warm climate, space and access to saline water and CO<sub>2</sub> sources [11–13].

To guide high performance systems development, the key purpose of this study was to analyse the response of these systems to typical operational light and temperature conditions in terms of photon conversion efficiency (PCE), areal ( $P_{areal}$ ) and volumetric biomass productivity ( $P_{vol}$ ) as well as final volumetric biomass yield ( $Y_{vol}$ ). The trials were conducted in batch mode and were based on the use of previously optimised media and CO<sub>2</sub> sufficiency [7].

## 2. Material and methods

The trials were conducted at the Solar Biofuels Research Centre (SBRC, [www.solarbiofuels.org/sbrc](http://www.solarbiofuels.org/sbrc)) which provides advanced system testing facilities in a subtropical climate (University of Queensland, Brisbane, Australia). To facilitate comparison PBR arrays were adjusted to have similar illuminated surface area to footprint ratios using PBR dummies (Supplementary Table 2).

### 2.1. Microalgae

Due to legislative environmental restrictions, trials were conducted with two local isolates which were characterised according to [7]. The reference (REF) strain was *Chlorella sorokiniana* (i.e. isolate 12\_A9 [7]) which has been extensively used in other performance studies [14–17]. For details see Supplementary Fig. 1 [20–22]. The second strain was a ‘high performance’ (HP) *Chlorella* sp. (i.e. isolate 11\_H5, [7]) and selected on the basis that it was one of the top two performing strains in terms of growth rate and yield in microwell and flask trials under lab conditions [7] and preliminary outdoor HRP experiments.

### 2.2. Pre-culture preparation

Individual colonies of each species maintained on Tris-acetate-phosphate (TAP) [18] agar plates ( $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , 23 °C) were inoculated into liquid TAP medium under constant illumination (10 mL,  $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , ~25 °C). The culture volume was then gradually increased to 4 L through regular subculture in flasks (TAP medium) before being used as inoculum for a 20 L pre-sterilised cultivation bag (Pure Biomass, USA) (continuous  $310 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , ~25 °C using cool white fluorescent lights (Philips, TL-D 58W/840), room temperature (RT), ~0.5 L min<sup>-1</sup> air sparging, pH 7). Next the hanging bags were moved outdoors to adapt to natural conditions for 2 days (light intensity, day/night cycles, temperature fluctuation) until an optical density at 750 nm ( $OD_{750}$ ) of ~3 was reached. The season for both cultivations was late spring with a mean daily temperature range of 15.8–37.8 °C and 15.3–30.1 °C and a mean solar irradiation of 23.26 and 23.68 MJ m<sup>-2</sup> d<sup>-1</sup> (daily solar irradiance values from Bureau of Meteorology, BOM) for the cultivation of the REF strain and the HP strain respectively.

To increase the inoculum volume for pilot scale trials, the REF strain was next inoculated into a 1.5 m high flat panel PBR (300 L) using strain-specific optimised media [7] and grown to an  $OD_{750}$  value of 2.8. The culture was then inoculated into HRP, flat panel PBR (FP) and tubular PBR systems to an equivalent starting  $OD_{750}$  of 0.38. This strategy was designed to yield cultures in late exponential phase to exploit the dual advantage of adequate cell density and reduced lag phases upon inoculation into the pilot scale cultivation systems.

For the HP strain trials, two low flat panel PBRs (110 L) were inoculated with 10 L of the hanging culture bag culture using strain-specific optimised media [7]. After ~1 week the two cultures were mixed in a HRP system to establish a uniform starter culture. The cultures were then inoculated into HRP, flat panel PBR and tubular PBR systems to an equivalent starting  $OD_{750}$  of 0.184.

For the comparative trials all cultivation systems were inoculated simultaneously in the evening to reduce light induced stress and to allow cultures to equilibrate before sunrise. Each culture was pH controlled (pH 7, +0.2/–0.5) using ammonium hydroxide (8% v/v) as alkali and CO<sub>2</sub> (1% CO<sub>2</sub> and 99% air mixture) to acidify the medium. See Supplementary material section ‘Microalgae cultivation systems’ for additional detail on each system and its control.

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